

Remarks

Claims 1-12, filed on December 2, 2005 with the Amendment after Final, remain in the application. Amendments have been made herein to claims 1 and 3 in consideration of the comments of Examiner Delacroix-Muirheid in the interview on February 7, 2006.

The Examiner proposed that the word "prevention" should be deleted from the claims. The word "prevention" in the previously presented claims 1 and 3 identify a specific type of tumor cell. In the context it is used, it refers to those tumor cells that employ the Hh/Smo signaling for inhibition of differentiation and/or for inhibition of apoptosis, and this is clear to a person skilled in the art. However, considering the comments made in the interview on February 7, 2006, the word "prevention" has been replaced with "inhibition" in the currently amended claims 1 and 3.

Several other claim modifications were proposed by the Examiner, but after consideration by the Applicants have not been seen to be necessary or appropriate and so have not been made at this time.

Deletion of "... for prevention and/or apoptosis" from the preamble of claims 1 and 3 was suggested in the interview. The applicants consider such a deletion to be inappropriate. This wording defines the tumor cells that are subjected to the invented method with precision. When it is removed, definition of the subject tumor cells becomes less precise. For example, numerous scientific publications antedating not only this application but also the earliest priority of US Patent 6 291 516 B1 (cited in the Office communication of 03/25/2005) show that Hedgehog/Smoothed (Hh/Smo) signaling may be used by tumor cells also for proliferation (e.g., *Fujita E et al, Biochem Biophys Res Commun* 1997;238:658-664; a copy is enclosed). Applicants do not claim treatment which merely inhibits proliferation. The current wording is therefore seen to improve the clarity and precision of claimed subject matter. Accordingly it is submitted that the suggested amendment is not appropriate and the proposed amendment has not been made.

As for the suggestion made in said interview for changing the word "sufficient" in the claims 1 and 3 to "effective," if made, such a change would not seem to affect the scope of the claims. However, in the opinion of the applicants, "a sufficient quantity" of a compound is a more clear term than "an effective quantity." It should be noted that a search in the USPTO patent database on March 3, 2003, reveals that from 1996 to the present, the term "sufficient quantity" appears in the claims of 2913 US patents (Search query: ACLM/"sufficient quantity"). Similarly, the equivalent term "sufficient amount" appears in the claims of 9147 patents. Therefore the term

"sufficient quantity" is seen to be well understood and does not raise any ambiguity. Accordingly the applicants have not adopted a replacement of "sufficient" with "effective."

It was also proposed by the Examiner to add the word "pathway" after "Hedgehog/Smoothed signaling" in claims 1 and 3. Upon consideration of this proposal, however, the applicants believe the shorter term is preferable for the sake of brevity and because it fully conveys the meaning (the term is used and defined in the specification).

It is respectfully submitted that the claims presently on file fulfill all the requirements of patentability (please see the "Remarks" in the Amendment after Final filed December 2, 2005). However, considering the delay in the allowance of claims, we have made a search for the possibility of other applications to the PTO with claims or disclosure directed to a similar subject matter. A number of such applications have been identified and they are listed in the accompanying IDS form. Some of these patent applications have disclosure that include the uses of various inhibitors of Hh/Smo signaling for affecting functions of the transcription factors that are the targets of said signaling (principally Gli-1, Gli-2, Gli-3) so as to affect the proliferation or differentiation or survival of target cells for treating various diseases that include cancer and psoriasis. Another group of these applications disclose the uses of various inhibitors of Hh/Smo signaling for inhibiting angiogenesis for treatments of various diseases that include cancer and psoriasis. Not all of these documents have been fully studied at this time. However, US 2004/0110663 (filed August 29, 2003) and US 2005/0112707 (filed August 31, 2004) have disclosures which appear to be generally representative and to which therefore it is believed particular comments and comparison with the Applicants' invention will contribute to a timely decision on the patentability of the application. The following comments are therefore submitted without representation that these documents in all aspects represent the most pertinent of the documents listed in the accompanying IDS.

U.S. Patent Application 2004/0110663 A1

The earliest priority claimed in the application 2004/0110663 A1 is to the U.S. provisional application No. 60/240564 (filed on October 13, 2000). The other claimed priority documents were filed after the filing of the Applicants' international application (PCT/TR01/00027, filed July 2, 2001), which fully supports the current claims and to which the present application is therefore entitled benefit. Accordingly the comments herein are directed primarily to the contents of application 60/240564.

Claim 1 in 60/240564 is directed to "a method of inhibiting unwanted cell *proliferation*" comprising determining a sign of Hedgehog signaling activity in the subject cells and contacting

said cells "with an effective amount of a hedgehog antagonist" so as to cause decrease of cell proliferation. Claim 3 of 60/240564 is dependent on claim 1 and reads "A method of claim 1, wherein said unwanted cell *proliferation* is cancer."

Since the claims in this priority document are directed to the inhibition of cellular proliferation which, according to claim 3, may be used for treatment of cancer, it appears appropriate to evaluate if the disclosure and claims of 60/240564 can anticipate or make obvious the subject matter of present application. It would also be important to determine if the prior art methods would be capable of creating the technical effects described by the applicants; i.e., whether or not the technical effects of the inductions of the apoptosis and/or differentiation of tumor cells are inherently present in the prior art uses of cyclopamine or a derivative thereof.

As detailed in our previous response (filed on June 24, 2005), cyclopamine and other specific inhibitors of Hh/Smo signaling were evaluated in prior art for their effects on cellular proliferation, differentiation and survival. The prior art had found an inhibitory action of Hh/Smo signaling inhibitors on the differentiation of various cell types and it was explicitly disclosed in the studies employing cyclopamine or a derivative thereof that "*significant ... cell death ... was not observed, even at the highest doses of compounds*" that completely inhibited the Hh/Smo signaling in transformed cells and resulted in inhibition of proliferation. The cells that were studied had a loss of functional Ptc and, consequently, had constitutive activation of Hh/Smo signaling which is common in tumors.

All prior art documents that claim the use of cyclopamine or a derivative thereof for tumor treatment control the amount to be administered to a patient for the obtaining of described therapeutic result (WO 99 52534 A1, page 59, lines 15-20; WO 00 41545 A2, page 63, paragraph 4; WO 01 27135 A2, page 74, lines 23-28; US application 60/240564, page 114, paragraph 4) and the expected as well as described technical effect and therapeutic result therein are the inhibitions of tumor cell proliferation and of tumor growth (please see also the "Remarks" in our Amendment after Final). Thus, *while it is true that the prior art publications also disclose a method of treating tumors by administration of an effective amount of a Hh/Smo signaling inhibitor, the expected and described technical effects and therapeutic outcome are clearly different between the prior art and present application.* Further, comparative investigations were also carried out in prior art where the influence of cyclopamine on the viability of normal and transformed cells (including the well-known tumorigenic cells) was evaluated (*Dunn MK et al, Developmental Dynamics* 1995;202:255-270, a copy is enclosed). Experimental findings were described and it was disclosed explicitly that the same concentration of cyclopamine that causes extensive death of the normal cranial neural crest cells does not cause death of the transformed cells and that the latter

cells "...are resistant to cyclopamine-induced cell death" (Dunn MK *et al*, *ibid*). Thus, the prior art disclosures based on the evaluations of the effects of cyclopamine or a derivative thereof on cellular viability/death are simply the opposite of what is described in this application. Moreover, this is true not only with regard to the utilization of said compounds for inducing tumor cell death but also concerning the relative susceptibilities of normal versus tumor cells to the causation of cell death by use of said compounds. Accordingly, the causations of tumor cell apoptosis and/or differentiation by use of cyclopamine or a derivative thereof, while sparing normal cells, are not only unknown in prior art but also highly unexpected and surprising (unobvious) in view of prior art. The fact that cyclopamine or a derivative thereof were actually applied in prior art on transformed cells and were found not to cause cell death at concentrations causing inhibition of the Hh/Smo signaling and of cellular proliferation is evidence that the inductions of the apoptosis and/or differentiation of tumor cells are not inherently present in the prior art uses of said compounds. We had pointed in this respect (in our previous response filed on June 24, 2005) that the inhibition of cellular proliferation does not necessarily cause induction of cellular differentiation or apoptosis and there are well known situations where cellular proliferation for one or more cycles is absolutely required for inducing differentiation or apoptosis (the fundamental reasons were also mentioned). Studies that have been published after the filing of present application document that, indeed, the responses of cells to the inhibition or upregulation of Hh/Smo signaling in terms of differentiation or apoptosis or proliferation depend on the cell types, their environment as well as on the means, extent and duration of the inhibition or upregulation of Hh/Smo signaling and confirm that Hh/Smo signaling inhibitors can be used for causing tumor cell differentiation and/or apoptosis (*Celso CL et al, Development* 2004;131:1787-1799, *Hutchin ME et al, Genes & Development* 2005;19:214-223, *Sanchez P et al, Mechanisms of Development* 2005;122:223-230) (copies of *Hutchin ME et al* and *Sanchez P et al* are filed herewith; a copy of *Celso CL et al* was filed previously).

The experimental findings described by *Sanchez et al* (*ibid*) provide further confirmation that *a dosing schedule of cyclopamine causing significantly inhibited tumor cell proliferation as well as inhibited tumor growth in vivo does not necessarily cause a significant induction of tumor cell apoptosis*. Similarly, *Hutchin ME et al* (*ibid*) described experimental conditions under which *the inhibition of Hh/Smo signaling in tumor cells led to the inhibition of proliferation to a level where no proliferating cells could be detected. Under the same conditions, however, the tumors continued to have viable tumor cells and these proliferation-inhibited tumor cells resumed proliferation upon discontinuation of the inhibition of Hh/Smo signaling*. Furthermore, *majority of the tumor cells that were subjected to a long-term inhibition of Hh/Smo signaling and to the*

inhibition of proliferation were not induced to differentiate. Still further, when the same Hh/Smo signaling-inhibited tumor cells were provided with an inductive environment, the tumor cells were found to differentiate and to give rise to numerous differentiated lineages that could participate in the formations of normal tissue structures (*Hutchin ME et al, ibid*).

Thus, whereas the prior art had no demonstration or unambiguous disclosure that cyclopamine or another specific inhibitor of Hh/Smo signaling can be used for inducing apoptosis and/or differentiation of tumor cells and pointed rather in the opposite direction, studies published afterwards confirm that inhibition of Hh/Smo signaling can be employed for obtaining said technical effects. Furthermore, it is clear that the inductions of tumor cell apoptosis and/or differentiation by use of cyclopamine or a derivative thereof are not inherent in the prior art uses of said compounds.

Sanchez P et al (*ibid*) have evaluated influence of the administration of cyclopamine on Hh/Smo signaling activity, proliferation and survival of tumor cells both in vitro and in vivo. In vivo, cohorts of mice having cerebellar tumors with activated Hh/Smo signaling were injected with cyclopamine or vehicle alone. A dosage of 10 mg cyclopamine per kg body weight was administered systemically once every other day for a month. The cyclopamine-treated and vehicle-treated animals were then sacrificed and their brains compared. Whereas the tumor-bearing cerebellar volume in vehicle-treated mice was increased to nearly 175 % of normal mice, tumor-bearing cerebellar volume in the cyclopamine-treated mice was increased to only about 10 to 20 % of normal (*Sanchez P et al, ibid, FIG. 3*). Comparative analyses showed that Hh/Smo signaling activity was inhibited in the cyclopamine-treated mice. Analyses of proliferation showed marked inhibition of tumor cell proliferation in the cyclopamine-treated mice whereas the analyses of tumors for apoptosis revealed no significant differences between the vehicle-treated and cyclopamine-treated mice (*Sanchez P et al, ibid, FIG. 3*). *Sanchez P et al* (*ibid*) indicate further that the induction of tumor cell apoptosis in vitro is also dependent on the quantity and duration of application of cyclopamine.

In comparison to the study of *Sanchez P et al*, the dosage of cyclopamine in our treatments of skin tumors can be calculated to provide a much greater concentration of cyclopamine around the tumor cells. While detailed published data is not available at this time on the volume of distribution and pharmacokinetics of cyclopamine, it can be calculated that the peak concentration that a dosage of 10 mg of cyclopamine per kg body mass can initially provide would not be more than about 25 μ M, assuming homogenous distribution. On the other hand, in the skin tumors exemplified in our application, a cream preparation that contained cyclopamine at a concentration of 18 000 μ M was applied directly onto tumors (ranging in size from about 4X3.5 mm to about

8X10 mm) in volumes of about 10 to 25 μ l per application. Considering the estimated overall size of tumors, the cyclopamine concentration around the tumor cells in our treatments would be magnitudes greater. In addition, the more frequent administration of cyclopamine in our case (every 3 to 4 hours) would provide lesser fluctuation of cyclopamine concentration around the tumor cells in comparison to the administration of cyclopamine every other day. As demonstrated, the dosage we used provided causation of massive apoptotic activity in the treated tumors and provided rapid decrease of size and disappearance of the tumors while sparing normal tissue cells. In addition, the tumor cells were shown to be induced to differentiate with unprecedented efficiency. The monoclonal antibody Ber-EP4, known to bind to the untreated tumor cells and to the normal tissue cells having stem/progenitor cell features, was shown to cease to detect the cyclopamine-treated tumor cells. In addition, a subpopulation of the same tumor cells were shown to have undergone differentiation to a more advanced stage detected by the *Ulex Europaeus* lectin type 1. It is emphasized in the specification that, whereas the tumor response to optimal concentrations of cyclopamine is rapid, suboptimal concentrations could not induce the differentiation and apoptosis of tumor cells (optimal conditions for attaining described technical effects were exemplified with independent tumors in a directly relevant setting, i.e., the actual treatment of human tumors in vivo).

In summary, the presently filed claims are directed to a previously unknown and unexpected subject matter and it is also clear that the technical effects and therapeutic outcome that are described and claimed are not inherent in the prior art uses of cyclopamine or a derivative thereof.

Other relevant disclosures of the US applications 60/240564 and 2004/0110663 A1, and our comments thereupon, are as follows.

Both applications 60/240564 and US 2004/0110663 A1 disclose and claim determination of gli gene products in cells as a measure of Hh signaling activity. However, numerous scientific publications in prior art, starting with the cloning and description of the *Drosophila ci* gene as the homologue of mammalian gli genes (*Orenic TV et al, Genes & Development* 1990;4:1053-1067), had established the gli 1, gli 2 and gli 3 as the major targets and mediators of Hh/Smo signaling (cf, e.g. the references in *Stamatakis D et al, Genes & Development* 2005;19:626-641; a copy was filed with our response filed on June 24, 2005). Therefore determining gli gene products as a measure of Hh/Smo signaling activity in a cell has been well known in prior art and is fully obvious to the skilled person.

The application 60/240564 describes on pages 132-133 (Example 3) experiments where cyclopamine was applied onto the cells exhibiting constitutive activation of Hh/Smo signaling

because of loss of a functional Ptc. The latter is well known to occur in many tumor types. It is disclosed explicitly in 60/240564 that said application of cyclopamine caused inhibition of the Hh/Smo signaling but caused no cell death (cf, page 133, paragraph 2). Besides the disclosures based on the experimental evaluation of the effects of cyclopamine on cell survival, the 60/240564 has speculative disclosures, that are also self-contradicting, concerning the influence of cyclopamine or another specific inhibitor of Hh/Smo signaling on cell survival. Thus, 60/240564 discloses on page 97, penultimate paragraph that subject method can be used for causing apoptosis and it discloses on page 109, last paragraph that "...because a hedgehog antagonist will often be cytostatic ... rather than cytotoxic", the subject method can be used for preventing apoptosis. The disclosures on page 100, paragraphs 3 and 4 also state that subject inhibitors can be used for enhancing survival of cells, including the neoplastic cells. Another disclosure on cell survival exists on page 90, penultimate paragraph where it is stated that "Another aspect of the present invention relates to methods of modulating a differentiated state, survival and/or proliferation of a cell". Considering that "modulation" may mean upregulation or inhibition and that these are the precise opposite of each other, there is no enablement or clarity in said disclosure. ***Thus, there is no enabling or unambiguous disclosure in 60/240564 that cyclopamine or another specific inhibitor of Hh/Smo signaling can be used for inducing tumor cell apoptosis.*** Rather, the experimental findings described therein teach to the skilled person that the uses of cyclopamine that cause inhibition of Hh/Smo signaling do not cause cell death in the cells exhibiting constitutive activation of Hh/Smo signaling.

The patent applications that are specifically referred to on page 29, paragraph 1 of 60/240564 also describe experiments that inhibition of Hh/Smo signaling by cyclopamine or a derivative thereof do not cause cell death in the cells lacking a functional Ptc. It should also be noted that the claims in 60/240564 are directed to an inhibition of cancer cell proliferation (claims 1 and 3) and it is the anti-proliferative effect of Hh/Smo signaling inhibitors that is contemplated therein for treatment of cancer (page 132, paragraph 2).

The application 60/240564 discloses that "given that hedgehog promotes angiogenesis, hedgehog antagonists may be useful in cancer treatment" (page 91, the first and last paragraphs). It has been well known in prior art, however, that the signaling initiated by the Hedgehog proteins stimulates the formation of new blood vessels and the inhibition of said signaling by various means inhibits angiogenesis (please see the enclosed review of related scientific literature and the references therein).

Both 60/240564 (page 97, paragraph 1) and 2004/0110663 A1 (paragraph 0620) specifically disclose that the subject method and antagonists can be used for treating multiple

sclerosis. However, it is well known that Hh/Smo signaling is required for the remyelination of neurons in multiple sclerosis as well as in the other related demyelinating diseases of nervous system. Consequently, it has been demonstrated that the administration of a Hh/Smo signaling upregulator provides therapeutic effect in said diseases and that Hh/Smo signaling antagonists are expected to cause rather a worsening of said diseases (cf, e.g. *Mastronardi FG et al, The Journal of Immunology* 2004;172:6418-6426).

There are also other inoperable disclosures in 60/240564 and in 2004/0110663 A1. Numerous scientific publications in prior art and afterwards have demonstrated that, in terms of cellular proliferation, upregulation of Hh/Smo signaling can be stimulatory and the inhibition of said signaling can be inhibitory for certain cell types but different cell types in the same organism can respond very differently, even in the opposite directions to the same modulation of Hh/Smo signaling (cf. the enclosed review of related scientific literature and the cited publications). For example, systemic administration of cyclopamine to mice has been shown to cause a marked stimulation of the proliferation of gastric epithelial cells whereas the proliferation of intestinal epithelial cells in the same animals is inhibited significantly (*van den Brink GR et al, Gastroenterology* 2001;121:317-328; a copy is enclosed). Furthermore, *Vila G et al (Journal of Clinical Endocrinology & Metabolism* 2005;90:6687-6694; a copy is enclosed) have analyzed a large number of human pituitary tumors and described that the Sonic Hedgehog protein inhibits the proliferation of certain pituitary tumor cells and that the proliferation of certain pituitary tumor cells is stimulated by cyclopamine.

Application 60/240564 discloses on page 13, penultimate paragraph that "*An effective amount of, e.g., a hedgehog antagonist, with respect to the subject method of treatment, refers to an amount of the antagonist in a preparation which, when applied as part of a desired dosage regimen brings about, e.g., a change in the rate of cell proliferation and/or the state of differentiation of a cell and/or rate of survival of a cell according to clinically acceptable standards for the disorder to be treated*". The same disclosure is repeated in 2004/0110663 A1 in paragraph 0111. The cellular proliferation, differentiation and survival/apoptosis of cells represent most basic life processes and "*bringing about a change*" in them "*according to clinically acceptable standards for the disorder to be treated*" is obviously desirable not only with Hh/Smo signaling antagonists but also with other medications and methods of treatment. The above-referred disclosures bring nothing new or enabling. Furthermore, it is well-established that the responses in terms of proliferation or differentiation or cell survival to the inhibition of Hh/Smo signaling depend very much on the cell types, their environment as well as on the means, extent and duration of inhibition. Also, the attaining of a therapeutic effect by the administration of a

Hh/Smo signaling antagonist is inherently unfeasible for some of the diseases claimed to be treatable in these applications (examples mentioned above). Thus, the quoted disclosures in 60/240564 and 2004/0110663 A1 clearly fail to be enabling and represent a mere desideratum.

In summary, there is nothing in the US Patent application 2004/0110663 A1 and its priority document that was filed prior to the filing of the Applicant's international application (PCT/TR01/00027) that can anticipate or make obvious the subject matter claimed in the present application.

U.S. Patent Application 2005/0112707 A1

This document, filed August 31, 2004 is not seen to be prior art to the present invention. Double continuation-in-part priority is claimed to 09/825,155, filed Apr. 3, 2001, which in turn is said to be a continuation of application 09/102,491, filed Jun. 22, 1998, now U.S. Pat. No. 6,238,876. Therefore application 09/825,155 can contain no new matter over that disclosed in to US 6,238,876. Inspection of US 6,238,876 reveals no reference to treatment with cyclopamine or derivatives thereof. All of the other applications to which priority is claimed postdate the July 2, 2001 priority date of the present application

U.S. Patent application 2005/0112707 A1 is seen to have disclosure and claims in some ways that overlap with those of the 2004/0110663 A1. It presents, however, more detailed and controlled experiments in the Examples section. A further difference of the disclosure of 2005/0112707 A1 from that of 2004/0110663 is that, when tumor cells are treated with a combination of a Hh/Smo signaling inhibitor and a known inducer of cell death, a greater proportion of the tumor cells will undergo cell death.

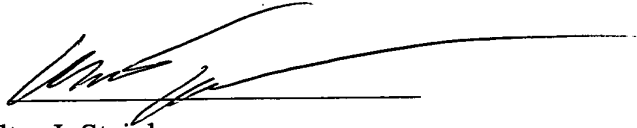
A noteworthy disclosure of 2005/0112707 A1 that would help evaluation of present application is found in paragraph 0370 and FIGURE 4. It is disclosed therein that the treatment of tumor cells with a sufficient amount of cyclopamine for a sufficient duration can cause death of 100 % of the tumor cells. It is shown further therein that, whereas the treatment of same tumor cells with cyclopamine for a shorter duration suffices to cause a marked inhibition of proliferation, the majority of inhibited tumor cells remain viable and resume proliferation upon the removal of cyclopamine from their environment. This adds *independent confirmation* that the invention as claimed herein is distinct, novel and non-obvious from the prior art disclosures pertaining to anti-proliferation uses of cyclopamine.

Conclusion

In view of the foregoing remarks and the amendments made, the presently filed claims are seen to recite subject matter that is enabled, definite and patentable over prior art. Accordingly the application is believed to be in condition for allowance. Early and favorable action is requested

Respectfully submitted,

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